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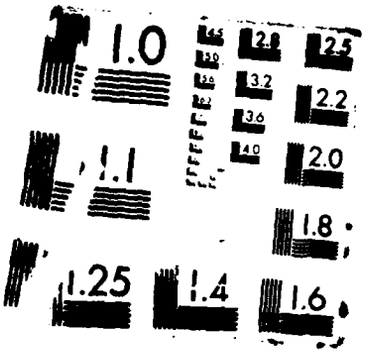
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An immunocytochemical study of the cerebellar cortex was carried out in order to determine the distribution and correlations of several neuroactive agents, vis., acetylcholine, taurine, gamma aminobutyric acid, and several peptides. Purkinje cells containing acetylcholine and its metabolic enzymes are most common in the flocculonodular lobe and they are randomly distributed without a clear pattern. In contrast Purkinje cells containing the enzymes glutamic acid decarboxylase (for GABA), or cystein-sulfinic acid decarboxylase (for taurine) or the peptide motilin are organized into microzones, longitudinal strips that alternate with strips containing other chemicals. Some Purkinje cells contain more than one neuroactive agent. These chemically characterized strips are closely matched with the location of mossy fibers representing the distal parts of the lower limbs. Keywords:

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Cytochemical Studies on Acetylcholine Synthesis and Metabolism
in the Vestibular Cerebellum
September 1, 1982 - August 31, 1986

Introduction:

This investigation was initiated in 1982 in order to explore the significance of acetylcholine and other neuroactive substances in the Purkinje cells of the cerebellar cortex. Initial plots of the location of Purkinje cells containing choline acetyltransferase showed that they were distributed randomly in the flocculonodular lobe in contrast to the cells containing the enzymes glutamic acid decarboxylase or cysteine sulfinic acid decarboxylase, or the peptide motilin, all of which are distributed in regular longitudinal strips. As the pattern of distribution of these other neuroactive agents appeared more precise and predictable than the pattern of the acetylcholine markers, it was decided to pursue this observation more closely to see whether it would correlate with the known longitudinal patterns of afferent fibers in the white matter of the cerebellum. The marker cysteine sulfinic acid decarboxylase, which catalyzes the rate limiting step in the formation of taurine from methionine or cysteic acid, was chosen for study in the rat. Plots of this marker showed that the distribution of Purkinje cells expressing the enzyme are nearly identical from one animal to the next. Spinocerebellar projections from the lower spinal segments, especially the lumbosacral enlargement, map onto the granular layer in longitudinal strips corresponding to the longitudinal strips of marked Purkinje cells. The regularity of the enzyme marker permitted the recognition of a medial to lateral pattern in the distribution of spinocerebellar afferents, that is, the cervical cord projects to the medial edge of each longitudinal zone or strip, the lumbrosacral cord projects to the lateral edge, and the thoracic cord to the region in between. The regular repeating pattern of the enzyme marker also suggests that the expression of the enzyme is location specific, and is not a transient metabolic property of the Purkinje cell dependent on circadian rhythms, stimulus traffic, and other contingencies, as suggested earlier.

In this report the work on acetylcholine will be summarized first and the bulk of the report will be devoted to work on other neuroactive agents, especially cysteine sulfinic acid decarboxylase, which has occupied the majority of the time since 1984.

1. Acetylcholine

a. In mice a large number of Purkinje cells in the flocculonodular lobe of the cerebellum display immunoreactivity to antisera against choline acetyltransferase and specific acetylcholinesterase, the metabolic enzymes for acetylcholine. Although preliminary results suggested that these immunoreactive cells were arranged in narrow

parasagittal strips (microzones), careful plots in serial sections failed to confirm such a pattern. The immunoreactive cells are dispersed throughout the lobe without condensation into bands.

b. Persistent efforts to combine immunocytochemistry for the two enzymes with elecron microscopy proved fruitless and this attempt was abandoned.

c. In order to ascertain the "birthday" of Purkinje cells with immunoreactivity to choline acetyltransferase, ten female mice were injected on gestation day 10, 11, 12, or 13 with tritiated thymidine and their progeny were examined with autoradiography after survival for varied periods after birth. The precursors of Purkinje cells in the mouse undergo their last cell divisions between the 11th and 13th days of gestation ("birthdays" of neurons). Immunoreactivity appears in Purkinje cells of the flocculonodular lobe between postnatal days 2 and 4. The intensity of the reaction increases until postnatal day 11 and remains at that level into adulthood. Immunoreactivity to specific cholinesterase follows the same time table. By combining autoradiography with tritiated thymidine and immunocytochemistry with antisera to choline acetyltransferase or acetylcholinesterase, or motilin, or glutamic acid decarboxylase, we obtained evidence that all four neuroactive agents appear at about the same time during postnatal days 2-4. Therefore, cells displaying a particular marker are not likely to be genetic clones from a single precursor.

c. Parallel immunochemical studies on specific acetylcholinesterase and choline acetyltransferase in the flocculomodular lobe indicate that both enzymes occur in the same Purkinje cells. Thus, in the vestibular cerebellum either enzyme is a reliable guide for the presence of acetylcholine production.

2. The traditional and still generally accepted view since the late 1960s is that throughout the cerebellar cortex all Purkinje cells fill the same role in cerebellar circuitry, exhibit the same function, and possess the same chemistry. All Purkinje cells are described as inhibitory and use gamma aminobutyric acid (GAB) as their transmitter. Yet, evidence accumulating over the past five or six years challenges this uniformitarian generalization. Not all Purkinje cells exhibit immunoreactivity to antisera against purified glutamic acid decarboxylase, the enzyme that catalyzes the rate limiting step in the synthesis of gamma aminobutyric acid. The cells containing this enzyme are arrayed in longitudinal strips alternating and partially overlapping with cells that contain markers for other neuroactive agents. A motilin-like substance appears in about 40 percent of Purkinje cells and these are also arranged in longitudinal strips or microzones. In addition, other markers, such as 5'-nucleotidase and immunoreactivity to a monoclonal antiserum known as Q 113, also appear in longitudinal strips.

This laboratory has selected for intensive study the marker cysteine sulfinic acid decarboxylase (CSDase) which catalyzes the rate limiting step in the synthesis of taurine, an amino acid which is more abundant in the cerebellum than anywhere else in the brain except the retina. Antisera against this enzyme provided to us by J.Y. Wu of the Hershey Medical Center, demonstrates again that Purkinje cells are chemically heterogeneous. In the rat Purkinje cells displaying immunoreactivity to this enzyme appear in longitudinal strips in the vermis and paravermis of the anterior lobe and again in the vermis of lobules VII and VIII of the posterior lobe. In the hemispheres, the flocculonodular lobe, and the vermis of lobules VI and IX, almost every Purkinje cell displays this marker. A similar pattern appears in the mouse, rabbit, and monkey.

a. The CSDase microzones in the rat have been the focus of our investigation for the past two years. The distribution of CSDase Purkinje cells is reliably and regularly repeatable in each animal. There is a midline strip consisting of a row of cells one to three cells wide. In the first three lobules it is only one cell wide, expanding to two or three cells as the strip advances posteriorly. Usually this strip does not have a representative in the most anterior face of the first folium. Symmetrically placed on either side of the midline and separated from the midline microzone by about 300 um is a pair of microzones about 4 or 5 Purkinje cells wide. This strip is also narrower in the first three lobules and widens gradually as it advances posteriorly. A second pair is symmetrically arranged an equal distance lateral to the first pair. This second pair is much wider, as many as 10 cells wide, and becomes more diffuse as it advances posteriorly. In lobule IV and V, a small intermediate microzone appears between the midline microzone and the first symmetrical pair of microzones. Still more laterally the marked Purkinje cells form almost continuous sheets from the edge of the paravermis into the lateral hemispheres. This pattern, as mentioned, is remarkably constant from one animal to the next. The pattern has been plotted onto maps and then converted into a digital record according to an image analyzing computer program in the laboratory of Dr. Richard Sidman in the Children's Hospital. The computer program produces a numerical, quantitative readout of the patterns, which allows detailed statistical comparisons to be made between animals. The mapping and conversion is very time consuming and is still in progress. Therefore we cannot report here the ultimate figures.

In order to search for correlations between the pattern of Purkinje cell microzones and the longitudinal strips of afferent fibers to the cerebellar cortex, one set of these fibers was labeled by injections of isotopically labeled methionine into the spinal cord of rats. The spinal cord was chosen because it was already known that spinocerebellar fibers are distributed in the anterior vermal and paravermal regions of the cerebellum, where the CSDase microzones are most obvious. Methionine labeled with ^{35}S was injected into the lower thoracic cord. After three or four days the animals were deeply anesthetized with chloral hydrate and perfused with Bouin's solution. Serial vibratome sections of the cerebellum were cut, treated according to the established procedure for

displaying immunoreactivity against CSDase antiserum and then mounted on slides, coated with NTB emulsion and exposed in light-tight boxes at 4 C for three to four weeks. The radioactively labeled methionine was incorporated into protein by nerve cells in the spinal cord and the resultant labeled protein was carried by axoplasmic transport orthogradely to the nerve endings of mossy fibers in the cerebellar cortex. There teminals are distributed in parallel, symmetrical longitudinal strips in the vermis and paravermis, according to a somatotopic pattern. When the injection site was located in the lower thoracic cord, the corresponding labeled mossy fibers formed strips lying just medial to the symmetrical CSDase microzones in the Purkinje cell layer. When the injection was located in the lower cervical and upper thoracic cord the corresponding labeled mossy fibers lay even more medial. When the injection site was located in the lower lumbar and sacral cord, the strips of labeled mossy fibers exactly matched the CSDase microzones of the Purkinje cells. Thus, the rostrocaudal somatotopy of the spinal cord segments is matched by a corresponding, repeated mediolateral zonation in the cerebellar cortex. This correspondence would not have been noticed without the reliable location of the CSDase microzones, identical in each animal. This material will be published together with the study of CSDase microzones.

From this investigation a number of conclusions can be drawn and some interesting speculations offered. 1. Purkinje cells, far from being uniform, are heterogeneous in their chemistry. 2. The overlapping but differing chemical microzone patterns indicate that many Purkinje cells bear a variety of neuroactive agents in coexistence. 3. At least for certain neuroactive agents, for example CSDase or taurine, their expression is location specific and continuous. This conclusion does not rule out transient or cyclic, quantitative variations in the expression of the agent in such cells. 4. The role of Purkinje cells with differing transmitter chemistry must be speculative in the present state of knowledge. The somatotopy of the spinocerebellar projection suggests that the Purkinje cells that receive a high proportion of their spinal input from the more distal parts of the lower limbs release taurine in their synaptic projection field, probably together with GABA. As Purkinje cells have a widely divergent axonal terminal field and each of the cells in the central nuclei receive input from a large number of overlapping Purkinje cells, the effect of these CSDase cells must be to change the relative weight of Purkinje cell activation, either to enhance or to diminish its effect, where this cohort of Purkinje cells is activated from lower spinal stimulation. It cannot be conceived of as exerting its effect alone; what it does is change the balance among the several input sources. There is some evidence that when GABA is released together with other inhibitory transmitters such as taurine, the inhibitory effect is potentiated, rather than merely additive.

b. Additional studies have been carried out on the distribution and characterization of catecholamine-containing cells in the hypothalamus of rats. These resulted in a careful map of these cells distributed throughout the medial parts of the hypothalamus (see publications).

3. In another region of the brain, the hippocampal formation, a mixture of GAD and various peptides have been found in the search for neurotransmitter candidates. As this formation is implicated in memory storage and in emotional control, the analysis of transmitters is important for both pharmacology and for understanding the physiology of this area. As examples of the findings, two may be cited here. The neuropeptides somatostatin and vasoactive intestinal polypeptide were localized in the retrohippocampal region. The two peptides were shown to occupy different layers in the cortex and to appear in different cell types. Somatostatin is principally located in the cells of the deeper layers, whereas the more superficial layers contain vasoactive intestinal polypeptide. The cells with the latter peptide resemble interneurons seen in Golgi preparations, while the somatostatin containing cells appear to be projection neurons (see publications).

4. A related neuropeptide, neuropeptide Y, has also been studied in the human hippocampus in both normal and Alzheimer's brain. The cells containing this peptide also appear to be small interneurons, posssible basket cells (see publications).

List of publications (Period: September 1, 1982 - August 31, 1985)

Book:

Chan-Palay, V. and Palay, S.L., eds. Coexistence of Neuroactive Substances. New York, John Wiley and Sons, 1984.

Papers:

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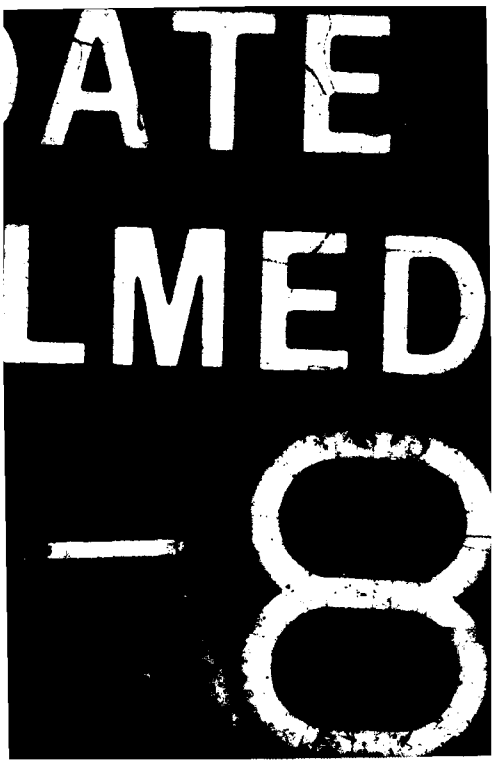
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